Expression of 1,25-Dihydroxyvitamin D_3 Receptors in Normal and Psoriatic Skin

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Increasing evidence suggests an immunoregulatory function of the potent steroid hormone 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) which has been successfully applied for treatment of psoriasis. The skin is both a site of production and a target of $1,25(OH)_2D_3$. In vitro, $1,25(OH)_2D_3$ inhibits proliferation and stimulates differentiation of keratinocytes.

We investigated the in situ expression of vitamin D-receptors (VDR) in normal and psoriatic skin by immunochemical methods. The VDR were visualized using the monoclonal antibody (MoAb) 9A7g to the VDR and the labeled avidin-biotin technique. Immunoreactivity was consistently confined to nuclei in all skin biopsies. In normal skin specimens (n = 10) VDR antigens were expressed in keratinocytes of all epidermal layers (except those of the stratum corneum) and in cells of the epidermal appendages. Double labeling experiments with MoAb to cluster-defined antigens indicated that melanocytes and approximately 75% of Langerhans cells exhibit $1,25(OH)_2D_3$ receptors in normal skin biopsies (n = 5). Depending on their localization in skin compartments 42–62% of $CD11b^+$ positive macrophages and 45–75% of $CD3^+$ T lymphocytes expressed VDR. Non-lesional psoriatic skin specimens (n = 8) revealed nearly identical staining patterns. Lesional psoriatic skin specimens (n = 8) exhibited a significant increase of VDR expression both in basal and suprabasal epidermal layers as measured by computer-assisted morphometry and showed a remarkable change of the immune cell pattern: the densitity and proportion of VDR positive T lymphocytes and macrophages were higher in the epidermal and the perivascular papillary loop compartment.

These in vivo findings strongly support the hypothesis that $1,25(OH)_2D_3$ modulates immune response and cell proliferation/differentiation in human skin. *J Invest Dermatol 97:230–239, 1991*

Vitamin D_3 is photochemically synthesized in the skin [1]. Its active metabolite 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) is formed by successive hydroxylation in liver and kidney and is responsible for the long known hormone function on maintenance of calcium homeostasis. $1,25(OH)_2D_3$ exerts its effects via binding to an intracellular receptor, present in target tissues [2,3]. The human $1,25(OH)_2D_3$ receptor (VDR) has recently been cloned [4]. Sequence comparisons demonstrated that this protein belongs to the superfamily of trans-acting transcriptional regulatory factors, which includes the steroid and thyroid hormone receptors and the retinoic acid receptor (for review see [5]).

It is now known that the skin itself is a target for $1,25(OH)_2D_3$. Functional expression of VDR, i.e., nuclear binding of $(^3H)1,25(OH)_2D_3$ in the skin of experimental animals, was reported by Stumpf et al [6] applying autoradiographic techniques. $1,25(OH)_2D_3$ is a strong inhibitor of proliferation and inducer of terminal differentiation of keratinocytes in culture [7]. Specific binding sites for $1,25(OH)_2D_3$ have also been demonstrated in vascular endothelial cells [8] and in cultured malignant melanoma cells [9]. However, data about its effect on human melanocytes and expression of the respective receptors [10,11] are still conflicting.

The discovery of VDR in monocytes and in activated but non-resting T and B lymphocytes suggests an immunoregulatory func-

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Abbreviations:

APAAP: alkaline phosphatase–mouse anti-alkaline phosphatase complexes
CD: cluster of differentiation
DAB: 3,3′-diaminobenzidine
$1,25(OH)_2D_3$: 1,25-dihydroxyvitamin D_3
DMSO: dimethylsulphoxide
FITC: fluorescein isothiocyanate
HE: hematoxylin and eosin
IFN-gamma: interferon-gamma
LC: Langerhans cell(s)
MoAb: monoclonal antibody
PBS: phosphate-buffered saline, pH 7.2
RT: room temperature
TBS: tris-buffered saline, pH 7.8
Tris: tris(hydroxymethyl)aminomethane
VDR: 1,25-dihydroxyvitamin D_3 receptor

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tion of $1,25(OH)_2D_3$ (reviewed in [12,13]). In vitro this hormone promotes the differentiation of monocytes/macrophages and enhances their effector functions, whereas it has down-regulatory effects on T-cell activation. These data suggest that $1,25(OH)_2D_3$ could play a role in the pathogenesis of psoriasis, a disease characterized by hyperproliferation and altered differentiation of keratinocytes and inflammation involving predominantly T cells [14,15]. Partial resistance of psoriatic fibroblasts or keratinocytes to the antiproliferative activity of $1,25(OH)_2D_3$ has been reported [16,17]. Recent clinical studies on the treatment of psoriasis with topical $1,25(OH)_2D_3$ [18] or analogues [19] were encouraging; other clinical trials are currently underway.

The aim of our study was to investigate VDR expression in normal and in psoriatic skin in vivo. We addressed the following questions. Which cells present in normal human skin express VDR in vivo? Do cells related to the skin immune system [20] represent potential targets for $1,25(OH)_2D_3$ as well? Does lesional or non-lesional skin of psoriatic patients exhibit any general defect or difference in VDR expression as compared to normal skin?

VDR were visualized by an immunohistochemical method based on a monoclonal antibody (MoAb) 9A7g to the VDR [21,22] and a labeled avidin-biotin technique (LAB) [23]. To immunophenotype VDR positive cells, we established a double labeling procedure for the simultaneous demonstration of those nuclear receptors and cluster defined (CD) cell membrane antigens [24].

MATERIALS AND METHODS

Skin Specimens Normal human skin was obtained from the legs of 10 patients (mean age 35.7 years, no history of skin disease) with informed consent. Lesional and non-lesional psoriatic skin (6-mm punch biopsies) was obtained from the arms or legs of 14 patients (mean age 48.0 years, chronic stationary psoriasis vulgaris, no treatment for at least 4 weeks). Lesional skin was taken from chronic plaques. Non-lesional skin was taken from the same site, but from clinically healthy skin adjacent to the lesion. All specimens were embedded in OCT-Tissue-Tek II (Miles Laboratories, Naperville, Illinois), snap frozen in melting isopentane precooled in liquid nitrogen and stored at −80°C.

Monoclonal Antibodies VDR were visualized with the MoAb 9A7g and the labeled avidin-biotin technique as described previously [23]. The MoAb 9A7g is a rat immunoglobulin (IgG2b) and was developed against purified chicken intestinal VDR [22]. MoAb 9A7g was shown to react equally well and specifically with mammalian VDR from a variety of tissues, but did not bind to glucocorticoid or estrogen receptors [21,25]. MoAb 9A7g recognizes both occupied and unoccupied VDR, the epitope being localized near the DNA binding domain.

For immunophenotyping of the epidermal and dermal cells commercially available mouse monoclonal antibodies against cluster defined [24] antigens of white blood cells were applied. These MoAb, their cluster definition, commercial source, working dilution, and the generally accepted cellular distribution of the respective CD antigens are listed in Table I.

Table I. Monoclonal Antibodies Used in the Double-Staining Procedure[a]

CD	MoAb	Main Cellular Reactivity	Dilution	Source
CD1a	Dako-T6	LC, dentritic cells	1:100	D
CD3	Leu4	T cells	1:100	BD
CD4	Leu3a	T helper/inducer, M, LC	1:100	BD
CD8	Leu2a	T suppressor/cytotoxic	1:100	BD
CD11b	IOM1	M, G, NK cells,	1:100	IT
CD15	ION1	G, (M)	1:10	IT
CD16	ION2	G, NK cells, (M)	1:50	IT
CD22	Dako-CD22	B cells	1:100	D
	Dako L26	B cells	1:50	D
CD25	anti-IL-2R	activated T/B cells/M	1:10	BD
	anti-HLA-DR	activated T/B cells, M, LC	1:100	BD
	anti-Vimentin	LC, melanocytes, leucocytes, fibroblasts, endothelial cells	1:50	A

[a] LC, Langerhans cells; M, monocytes/macrophages; G, granulocytes; NK, natural killer cells; D, Dakopatts; BD, Becton-Dickinson; IT, Immunotech; A, Amersham.

Immunohistochemical Staining Procedures

Preparation of Sections and Fixation: Serial sections (5 μm) were cut on a cryostat, mounted on glass slides, and kept in the cryostat at −20°C for up to 20 min. The glass slides were previously derivatized [26] by incubation with triethoxysilylpropylamin in DMSO and activated by 2% (v/v) glutardialdehyde. All sections were fixed in 3.7% formaldehyde in PBS (10 min, room temperature [RT]), rinsed in PBS (1 × 5 min), incubated in methanol (3 min, −20°C) and in acetone (1 min, −20°C) and transferred into PBS.

Staining of 1,25-Dihydroxyvitamin D_3 Receptors: The immunohistochemical method for the demonstration of VDR was applied as recently published [23]. It is described here in a condensed protocol: if not noted otherwise all of the incubation steps were performed in a moist chamber at RT covering the sections with 100 μl of the respective reagents. In each wash step, the sections were rinsed 2 times for five min in 500 ml PBS, prior to each next antibody incubation step.

To reduce non-specific staining, the slides were incubated with heat-inactivated 5% normal goat serum in PBS (20 min, RT), Avidin (15 min, RT), followed by a washing step, treated with Biotin (15 min, RT) and washed again. Avidin and Biotin were obtained from Vector (Camon, Wiesbaden, FRG).

The slides were then incubated (12 h, 4°C) with MoAb 9A7g at a dilution of 1:2000 or 1:4000 or as control with polyclonal rat IgG (Sigma, München, FRG) at similar concentrations. MoAb 9A7g and rat IgG had been diluted by PBS containing 1% bovine serum albumin (Sigma). After a washing step the sections were incubated with biotin-labeled goat anti-rat IgG (Jackson Immunoresearch/ Dianova, Hamburg, FRG) at a dilution of 1:400 (30 min, RT) and after an intermediate wash, incubated with streptavidin-peroxidase complexes (Jackson Immunoresearch) at a dilution of 1:400 (30 min, RT).

After a final washing, the sections were treated with 3,3′-diaminobenzidine–PBS- hydrogen peroxide (50 mg 3,3′-diaminobenzidine (Sigma), 100 ml PBS, 40 μl 30% H_2O_2) for 6 min. Those sections, which were not double labeled, were transferred into tap water, dehydrated, cleared in xylene, and mounted with Eukitt.

Double Staining Procedure: To identify the cells expressing VDR, a double staining procedure for the simultaneous visualization of VDR and CD antigens in the same section was developed:

First, VDR were stained with the labeled avidin-biotin method as described above, and second, the presence of various CD antigens was visualized using the MoAbs listed in Table I and the APAAP technique. VDR staining required formaldehyde fixation, which masked most of the CD antigens in tissue sections. Therefore a careful trypsin digestion was subsequently performed to reveal hidden antigenic sites. Morphometric control measurements were performed on paired sequential sections from three skin specimens stained with each of the MoAb listed in Table I after either formaldehyde/trypsin treatment or conventional acetone fixation. Both methods exhibited indistinguishable sensitivities for immunotyping. Further controls demonstrated that VDR staining (LAB) as the first step did not interfere with subsequent CD staining.

For the trypsinization, the slides were transfered from the tap water into a solution of 0.001% trypsin (from bovine pancreas type II, Sigma) in TBS and incubated for 10 min at 37°C. The digestion was stopped by washing in cold tap water (2 × 5 min) and TBS (2 × 5 min). As the next step, the sections were incubated with one of the mouse MoAb diluted in heat-inactivated normal rabbit serum. Pre-immune mouse ascites (Behring, Marburg, FRG) served

as negative control for the first mouse MoAb. After washing, the slides were incubated with rabbit anti-mouse (Dakopatts, Hamburg, FRG) 1:50 in 10% normal human serum in TBS (30 min, RT) followed by APAAP complexes (Dakopatts) 1:50 diluted in 1% BSA in TBS (1 h, RT). Substrate solution [0.2 mg/ml naphthol AS-MX Phosphate (Sigma)] in Tris pH 8.2 plus 0.24 mg/ml Levamisol (Sigma) and 1 mg/ml Fast Blue (Serva) was added for 20 min. The slides were finally rinsed in tap water and mounted with glycerol gelatin.

Staining Procedures for the Computer-Assisted Morphometric Analyses: In order to analyze the VDR expression in the epidermis of lesional compared to non-lesional psoriatic skin, serial sections of each biopsy were processed as follows: three sections were stained with MoAb 9A7g at a dilution of 1:2000. Two sections were stained with normal rat IgG at 500 ng/ml for the background control. After performance of the labeled avidin-biotin technique, these sections were (without double staining) dehydrated and mounted in Eukitt.

In the morphometric analyses we measured expression of VDR as VDR stained area versus total epidermal area. Earlier studies revealed that VDR expression occurs exclusively in the nucleus [23]. Because the percentage of nuclear area in relation to the total epidermal area may differ per se between lesional and non-lesional skin, we analysed in parallel the percentage of VDR positive nuclear area in relation to the total (hematoxylin stained) nuclear area. Therefore, three further serial sections of each biopsy were dyed in hematoxylin to stain the nuclei. To define a basal and a suprabasal layer in the epidermis for the computerized analyses, another serial section of each biopsy was incubated with MoAb BL7 against epidermal basal cells (Immunotech/Dianova, Hamburg, FRG) 1:5 and FITC-labeled goat anti-mouse IgG + IgM (Jackson Immunoresearch/Dianova) 1:20.

Morphometric Analyses

Enumeration of Double Stained Cells of the Skin Immune System: Enumeration was performed on sections of normal (n = 5), and on paired sections of lesional and non-lesional psoriatic skin (n = 8), which had been double stained for VDR and HLA-DR or VDR and one of the following CD antigens: CD1a, CD3, CD4, CD8, CD11b, CD15/CD16, CD22, CD25.

We relied mainly on the morphometric method developed by Bos et al [20] in defining different horizontal layers in each skin section and subdividing these horizontal layers into perivascular or other ("free") compartments. Thus, as schematically shown in Fig 1, five compartments were distinguished in each skin section: (I) epidermis, (II) perivascular papillary loop, (III) perivascular superficial plexus, (IV) perivascular reticular layer and perivascular adnex, (V) other ("free") papillary/reticular dermis.

The sections were analyzed by two independent investigators in a Standard-18-Zeiss microscope equipped with Plan-Neofluar ×16, ×25, ×40, ×63 objectives, and an eyepiece KPL ×10/18 with a 10 × 10 mm grid (Fig 1). For each staining and each compartment we analyzed three non-overlapping fields at high-power magnification (×400). The total number of CD^+ cells and the total number of VDR^+/CD^+ and VDR^-/CD^+ cells were counted in each field. The numbers of cells per field for each CD antigen and each compartment were added and used to calculate the cell number per 0.1 mm^2.

These values were used to determine the mean of the CD^+ cell density in each compartment and the mean percentage of VDR^+/CD^+ cells per compartment.

Computer-Assisted Morphometric Analyses of Epidermal 1,25(OH)$_2$D$_3$ Receptor Expression: The epidermal expression of VDR was measured quantitatively in lesional and nonlesional psoriatic skin of ten patients with an automatic and interactive image-analysis system. The whole system includes the following.

- A light microscope (type orthoplan; Leitz, Wetzlar, FRG) with a Leitz ×40 objective and color filters for contrast enhancement
- A black and white video camera (type TYK; Siemens, München, FRG) adapted to the microscope
- The automatic image analysis system IBAS I + II (Kontron, Eching, FRG) with a digitizing tablet for interactive control of image processing.

Images obtained with the video camera were digitized and further processed by contrast enhancement and shadow correction. Selection of positively stained structures was obtained by discrimination of their grey levels. This was done under visual control by interactively defining the thresholds. The epidermal area that was to be measured was outlined with an interactive device (Fig 2*A*). Two horizontal layers that had been defined by staining with MoAb BL7 were distinguished in the epidermis: a basal (Fig 2*B*) and a suprabasal layer (Fig 2*C*). The left and right borders of this area were set by the screen. The total of the outlined area (reference area) and the area of the positively stained structures (object area) was measured. For each section ten non-overlapping areas, five in each horizontal layer, equally spaced over the horizontal length of the biopsy, were examined by computer-assisted morphometric analysis. This was performed on paired sequential sections, one stained for VDR and the other with hematoxylin. When five areas in each layer were evaluated, interarea variation determined only up to 20% of the observed variance between patients. Reliability of the reading was tested by re-examination of randomly selected slides some weeks later giving low interassay variance (coefficient of variation <4%).

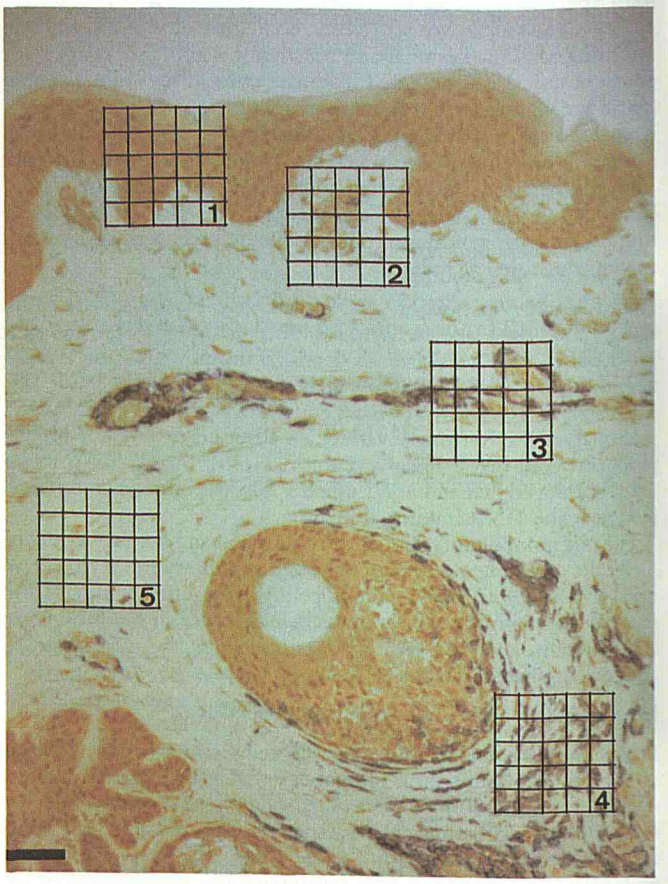

Figure 1. Morphometric analysis of VDR expression in immunocompetent cells: 5-μm section of normal human skin, double stained for VDR (brown labeled, 9A7g 1:2000, labeled avidin-biotin method) and $CD3^+$ cells (blue labeled, Leu 4 1:100, APAAP). *Rectangular grids*, the different compartments (I–V) in which $CD3^+$-positive cells were counted in the three non-overlapping fields at high-power magnification. Furthermore, in the same three fields, we counted the number of double-stained, i.e., $VDR^+/CD3^+$ cells. Note the strong expression of VDR in cells of the hair follicle and of sebaceous glands. *Bar*, 50 μm.

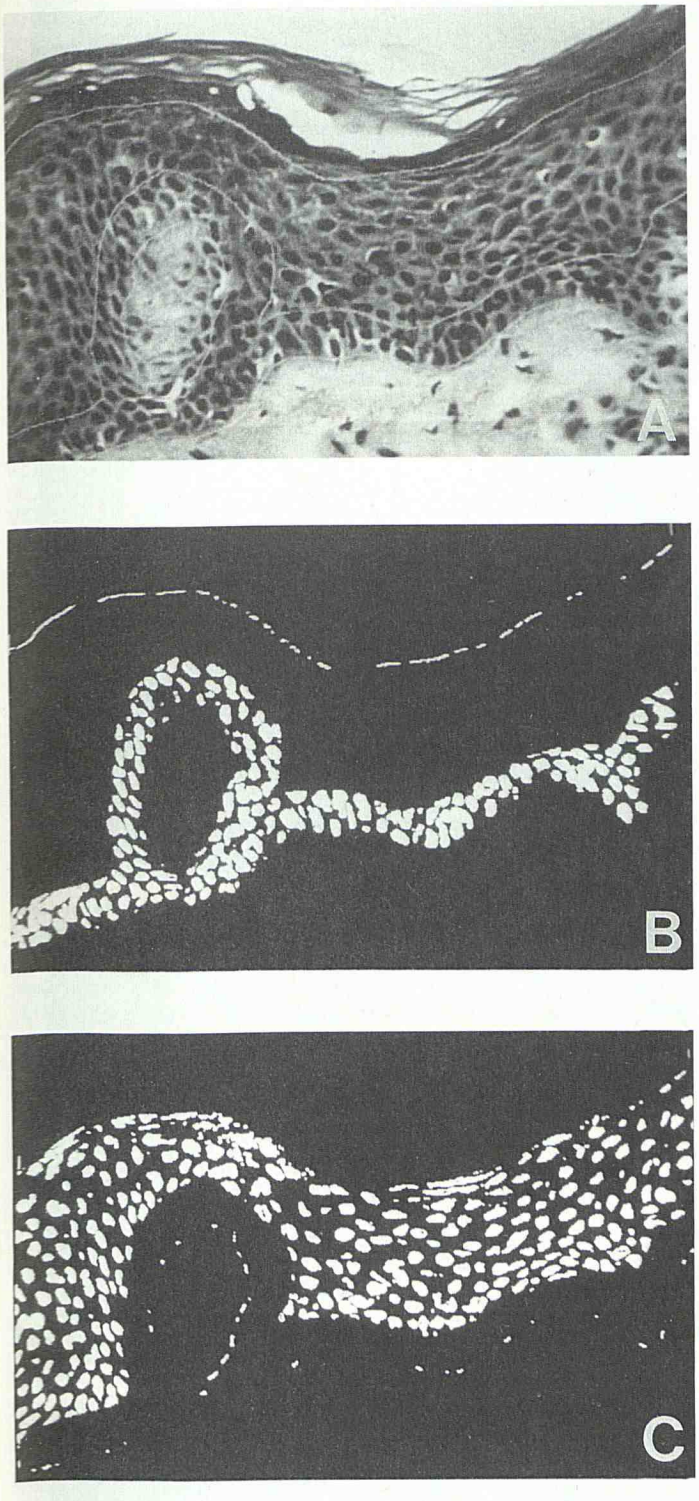

Figure 2. Computer-assisted morphometric analyses of epidermal VDR expression. *A:* Photograph of the monitor showing digitized image of normal human skin stained with MoAb 9A7g to VDR. *Thin white lines* were drawn with an interactive device to define the epidermal layers that were to be measured. Photograph of the monitor showing the interactively thresholded image *(B)* of the outlined epidermal basal layer and *(C)* of the outlined suprabasal layer, which were measured consecutively.

Homogenity of the staining and reading is also indicated by low SD between areas in one biopsy.

Thus, the following morphometric data were calculated for each biopsy:

$$\text{A VDR/A (\%)} = \text{area VDR/reference area VDR} \times 100\ (\%) = \text{VDR positive area in relation to total epidermal area}$$

$$\text{A hematoxylin/A (\%)} = \text{area hematoxylin/reference area hematoxylin} \times 100\ (\%) = \text{Nuclear (hematoxylin stained) area in relation to total epidermal area}$$

$$\text{A VDR/}A\ \textit{hematoxylin}\ (\%) = A\ \textit{VDR/A/A hematoxylin/A} \times 100\ (\%) = \text{percentage of VDR positive area in relation to nuclear (hematoxylin stained) area}$$

Statistical Evaluation The data obtained for the different skin biopsies were compared to each other by the Wilcoxon matched pairs signed rank test or the Wilcoxon two-sample test.

RESULTS

1,25-Dihydroxyvitamin D_3 Receptor Expression in Normal Human Skin The results of the VDR staining were qualitatively similar in all ten skin specimens. Positive staining with MoAb 9A7g was confined to the cell nuclei and absent when polyclonal rat IgG was used as a control (Fig 3*B*).

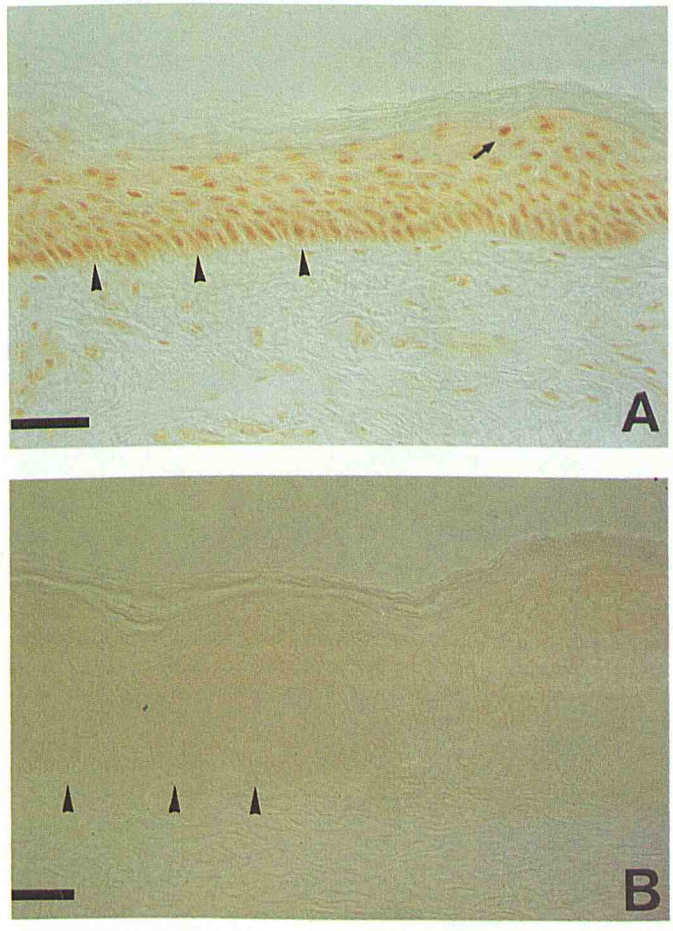

Figure 3. Epidermal VDR expression. *A:* 5-μm section of normal human skin immunolabeled with MoAb 9A7g to VDR (1 : 5000) (no phenotyping by MoAb to CD antigens). Keratinocytes in all epidermal cell layers show a nuclear staining. Note the more intense staining of basal cells *(arrowheads)* and single cells *(arrows)* in the upper stratum spinosum and stratum granulosum. *B:* Absence of nuclear staining in a sequential section incubated with rat IgG instead of MoAb 9A7g as control, basement membrane *(arrowheads)*. *Bar*, 40 μm.

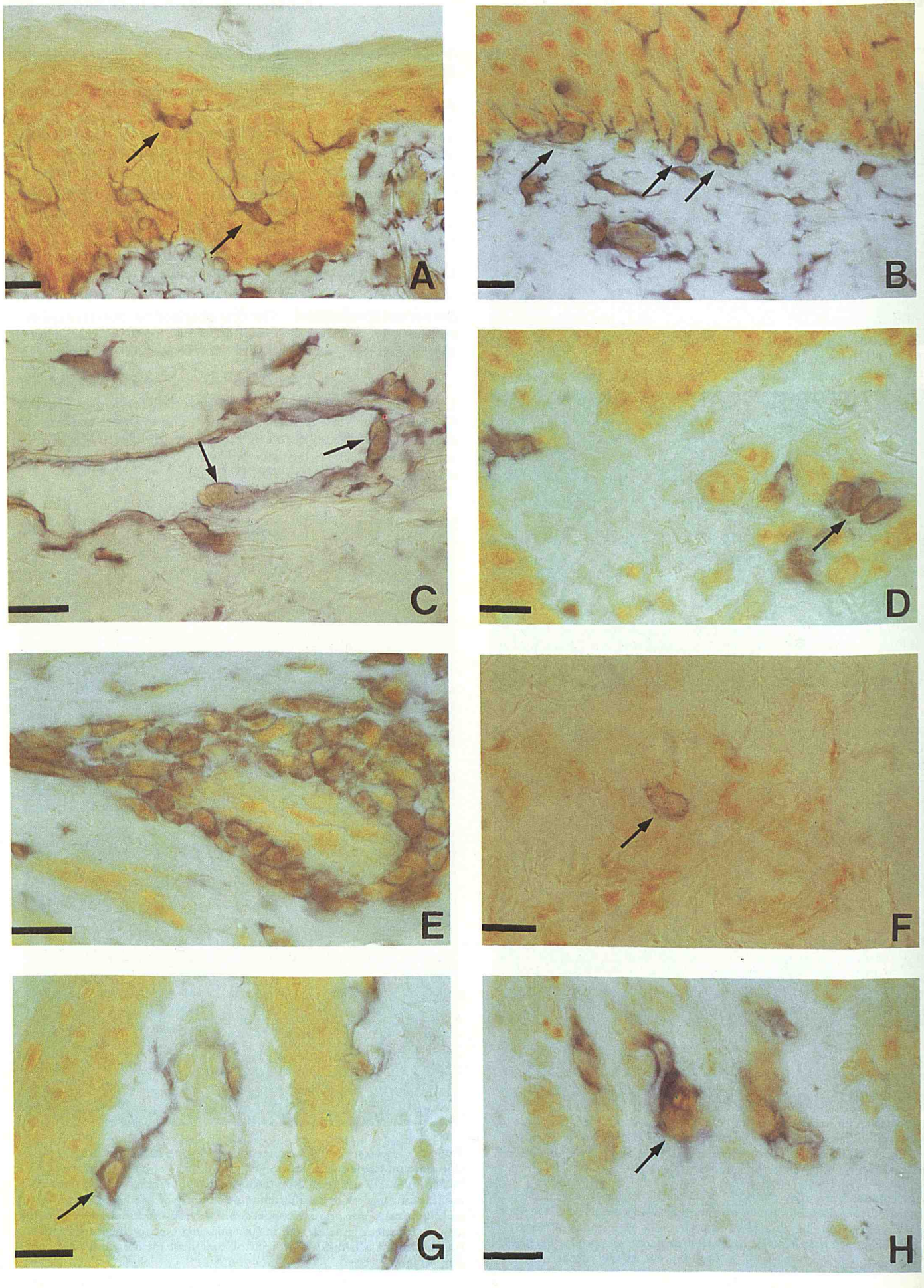
A
B
C
D
E
F
G
H

Table II. 1,25-Dihydroxyvitamin D_3 Receptor (VDR) Expression by Immunocompetent Cells in Different Compartments of Normal Human Skin

CD[a] VDR/CD[b]	Compartment[c] I	II	III	IV	V
$CD1a^+$ φ	39.7 ± 3.8	23.1 ± 7.6	100.1 ± 35.1	48.0 ± 23.7	0
$VDR^+/CD1a^+$	75.4 ± 6.5	55.6 ± 9.5	58.7 ± 14.0	58.4 ± 10.3	-
$CD3^+$ φ	3.6 ± 0.5	14.7 ± 5.1	86.0 ± 30.1	106.1 ± 95.1	0
$VDR^+/CD3^+$	75.0 ± 25.0	45.8 ± 6.7	63.8 ± 10.2	60.2 ± 15.0	-
$CD4^+$ φ	25.7 ± 3.0	44.5 ± 9.9	111.0 ± 28.8	193.8 ± 111.5	3.1 ± 0.6
$VDR^+/CD4^+$	63.6 ± 5.4	63.4 ± 4.9	60.8 ± 2.9	58.6 ± 7.1	56.0 ± 6.9
$CD8^+$ φ	0	7.0 ± 3.0	30.2 ± 15.9	24.0 ± 15.8	0
$VDR^+/CD8^+$		65.3 ± 5.4	68.2 ± 10.1	67.5 ± 9.0	-
$CD11b^+$ φ	0	71.5 ± 9.6	203.5 ± 58.0	191.9 ± 83.6	6.4 ± 2.9
$VDR^+/CD11b^+$		58.1 ± 5.6	42.4 ± 6.9	62.1 ± 8.9	54.8 ± 5.5
$CD15^+$ φ	0	9.5 ± 6.0	131.2 ± 117.4	6.6 ± 2.2	0
$VDR^+/CD15^+$		70.8 ± 23.6	77.8 ± 7.5	86.7 ± 18.9	-
$CD25^+$ φ	0	6.8 ± 4.0	21.8 ± 6.4	20.0 ± 11.6	2.3 ± 1.0
$VDR^+/CD25^+$		76.5 ± 14.8	83.3 ± 15.2	61.0 ± 17.3	75.0 ± 25.0
HLA-DR^+ φ	27.3 ± 6.8	125.8 ± 40.5	446.0 ± 101.6	200.9 ± 102.7	11.6 ± 10.7
VDR^+/HLA-DR^+	76.0 ± 7.4	57.3 ± 3.0	61.2 ± 4.7	56.8 ± 4.4	55.3 ± 2.8

[a] Expressed as number of CD^+ cells adjusted per 0.1 mm^2 section area.
[b] Expressed in percent of CD positive cells.
[c] I, epidermis; II, perivascular papillary loop; III, perivascular superficial plexus; IV, perivascular reticular dermis and perivascular adnex; V, "free," papillary, and reticular dermis; φ, mean ± SD; n = 5.

Epidermis The most prominent VDR staining reaction in all skin sections was seen in cells of the epidermis and of the epidermal appendages. As shown in Fig. 3*A*, VDR were expressed in the nuclei of all epidermal cell layers except the stratum corneum. In general, the staining intensity was stronger in keratinocytes of the basal layer than in upper layers. However, single scattered cells in the upper stratum spinosum and stratum granulosum were found to be heavily stained as well.

The double labeling experiments revealed that the majority of immigrant cells in the epidermis expressed VDR. *Langerhans cells* (LC) were identified by staining with MoAb to CD1a or HLA-DR or vimentin and by their characteristic morphology (Fig 4*A*). Seventy-five percent of the CD1a and 76% of the HLA-DR positive cells in the epidermis expressed VDR (Table II). In addition to LC (mainly located in the granular layer) MoAb to vimentin also marked a cell population, confined to, or below, the level of the basal cells. The location, the morphology, i.e., presence of smaller dentritic processes, the more ovoid nucleus, and the lack of expression of other CD antigens in sequential sections, suggested that these cells are *melanocytes*. As shown in Fig 4*B* these cells strongly express VDR.

Epidermal Appendages In comparison to all other cells labeled in the skin, the cells of the *pilosebaceous apparatus* contained the most heavily stained nuclei found in the normal skin section (Fig 1). In the *hair follicle* VDR were consistently most markedly expressed in the nuclei of the keratinocytes of the outer root sheath. In contrast, the labeling intensity of the cells of the inner root sheath was very heterogeneous, some nuclei staining strongly, others rather weakly. Similarly, the nuclei of the undifferentiated basal cells in the outer layer of the *sebaceous glands* stained more intensely than the more differentiated centrally located cells. As shown in Fig 5*A*, *smooth muscle* cells of the arectores pilorum revealed only a weak nuclear staining. *Eccrine sweat gland* cells (Fig 5*B*) were in their majority VDR positive: both the secretory coil, including the myoepithelial cells and all parts of the eccrine sweat duct were labeled.

Dermis The connective tissue always gave negative staining results. *Fibroblasts*, identified by anti-vimentin and by their shape, revealed either negative or very weak staining for VDR. In all biopsies, cells of the *cutaneous microvasculature* were VDR immunoreactive. In capillaries, arterioles and venules, endothelial cells (labeled with anti-HLA-DR or anti-vimentin [Fig 4*C*]) were consistently VDR positive. Pericytes or smooth muscle cells in the microvascular wall were clearly labeled as well.

Expression of 1,25-Dihydroxyvitamin D_3 Receptors in Cells of the Skin Immune System In order to investigate VDR in the skin immune system it was necessary to establish the type and location of immune cells in normal human skin. The density of the various cells in the different skin compartments is summarized in Table II. The majority of immunocompetent cells present in normal skin were T cells and macrophages. B cells could not be detected in normal skin. T cells were located almost exclusively around the superficial plexus and perivascularly in the reticular dermis or near the epidermal appendages. Most of these perivascular T cells belonged to the helper/inducer $CD4^+$ class with CD4/CD8 ratios ranging between 4 : 1 and 7 : 1. $CD11b^+$ macrophages were found in great density around the superficial plexus and perivascular in the reticular dermis, as were T cells. The "free" compartment, however, contained virtually no other cells of the immune system than macrophages. Granulocytes ($CD15^+/CD16^+$) were found in only three of the five cases, and in low numbers. The majority of the immunocompetent cells present in normal skin expressed HLA-DR antigen. HLA-DR^+ cells (activated T cells, macrophages, and veil cells) and indeterminate cells ($CD1a^+$, HLA-DR^+) were found predominantly around the superficial plexus.

Figure 4. Expression of VDR in immunophenotyped cells in normal human skin. 5-μm sections are first stained with rat MoAb 9A7g to VDR (MoAb 9A7g 1 : 2000, labeled avidin-biotin method, DAB as substrate) (brown labeling). The slides were subsequently incubated with mouse MoAb to CD antigens or Vimentin (APAAP procedure, Fast Blue as substrate). *A:* VDR-positive nuclei of Langerhans cells *(arrows)* stained with anti-vimentin MoAb (1 : 50). *B:* Melanocytes *(arrows)* stained with anti-vimentin MoAb (1 : 50) reveal strong nuclear expression VDR. *C:* Endothelial cells *(arrows)* (vimentin$^+$) in a dermal vessel show a nuclear reaction. *D-F:* T cells *(arrow)* around the capillary loop (D) or around a vessel near to a hair follicle (E) and immunophenotyped with MoAb to CD3. Note the VDR-positive staining. Single $CD8^+$ T-supressor cell *(arrow)* with positive nuclear staining (F). *G:* Macrophage ($CD11b^+$) *(arrow)* with long dentritic process, positively stained for VDR. *H:* Granulocytes ($CD15^+$) in the dermis, with even more intense labeling of the nucleus. *Bars*, 20 μm.

The proportion of those immunophenotyped cells that simultaneously expressed VDR varied among the different cell types (Tables II and IV). VDR expression within a given cell type did not significantly depend on its location in a certain skin compartment.

About 75% of $CD1a^+$ *Langerhans cells* in the epidermis and 56–58% of $CD1a^+$ *indeterminate cells* in the dermis expressed VDR. $CD3^+$ *T cells* stained for VDR in 57%, the *T-helper subset* ($CD4^+$) in 60%, and the *T-suppressor cells* ($CD8^+$) in 67%. $CD11b^+$ *macrophages* were found to be VDR positive in 56% and the low number of $CD15^+/CD16^+$ *granulocytes* in 75%. See also Fig 4*D–H*.

1,25-Dihydroxyvitamin D_3 Receptor Expression in Psoriatic Skin

Epidermis: No differences in epidermal expression of VDR were found comparing normal with non-lesional psoriatic skin (data not shown). In lesional psoriatic epidermis (Fig 6*A*), however, VDR expression was enhanced compared to non-lesional skin (Fig 6*B*).

The morphometric analyses gave the following results (Fig 7): the VDR-positive area in relation to total epidermal area ($A\ VDR/_A$) in non-lesional skin was $27.6 \pm 4.4\%$ in the basal layer, and $11.9 \pm 2.3\%$ in the suprabasal layer. This ratio was significantly lower ($p \leq 0.01$) than that in lesional psoriatic skin, where the mean $A\ VDR/_A$ was $33.4 \pm 4.4\%$ (basal), and $17.0 \pm 3.2\%$ (suprabasal).

To ensure that this result was not due to an altered relation between nuclear area and total epidermal area in lesional skin, hematoxylin-stained sections were analyzed in parallel. In the basal layer, the ratio of nuclear areas to the total epidermal area ($A\ hematoxylin/_A$) was significantly lower ($p \leq 0.01$) in non-lesional than in lesional skin. The means of the $A\ hematoxylin/_A$ were $34.2 \pm 3.6\%$ and $38.6 \pm 3.5\%$ respectively. However, no significant differences were measured between the relative hematoxylin stained nuclear areas ($A\ hematoxylin/_A$) in the suprabasal layer. The means were $25.2 \pm 3.2\%$ (non-lesional) and $23.5 \pm 3.2\%$ (lesional).

The proportion of VDR positively stained nuclei to total nuclei, i.e., VDR-positive area ($A\ VDR/_A$) in relation to the nuclear area ($A\ hematoxylin/_A$) was calculated as ($A\ VDR/_{A\ hematoxylin}$). $A\ VDR/_{A\ hematoxylin}$ was significantly increased in lesional psoriatic skin, in the basal (non-lesional 80.9 ± 9.8, lesional 86.4 ± 4.5; $p \leq 0.05$), and suprabasal layers (non-lesional 47.4 ± 7.9, lesional 72.9 ± 11.1; $p \leq 0.01$).

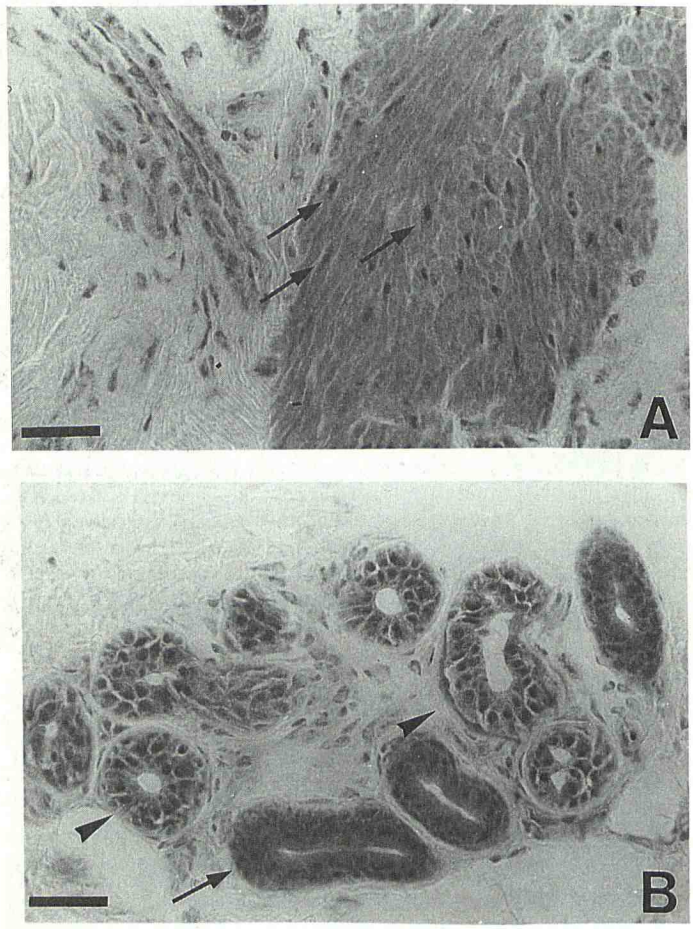

Figure 5. Epidermal appendages. 5-μm sections of normal human skin stained with MoAb 9A7g (1:2000) against VDR. *A:* Smooth muscle cells *(arrows)* of the arector pilorum show weak nuclear staining. *B:* Intense staining of eccrine sweat gland nuclei. Both secretory coil *(arrowheads)*, including the myoepithelial cells, as well as the duct *(arrow)* are labeled. Cytoplasmatic staining of duct cells is suggested to result from endogeneous biotin. *Bars*, 40 μm.

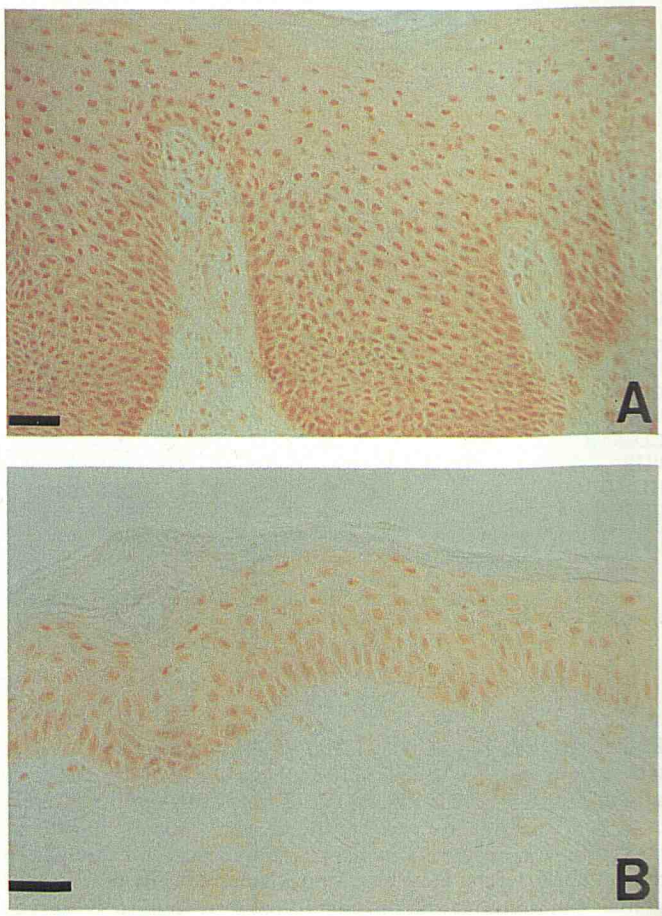

Figure 6. VDR expression in psoriatic epidermis. 5-μm sections of lesional *(A)* and non-lesional *(B)* psoriatic skin stained with MoAb 9A7g to VDR (1:5000). Note the enhanced expression of VDR in the lesional skin. *Bars*, 40 μm.

Expression of 1,25-Dihydroxyvitamin D_3 Receptors in Immunophenotyped Cells in Psoriatic Skin The following differences in the distribution of immune cells comparing lesional with nonlesional skin were found (Table III).

- A significant decrease ($p \leq 0.05$) of $CD1a^+$ Langerhans cells in the epidermis of lesional skin
- An increased epidermotropism of $CD11b^+$ cells ($p \leq 0.01$) and of $CD3^+$ T cells ($p \leq 0.01$) in lesional skin. The latter is partly due to an increased number of $CD8^+$ T cytotoxic/suppressor lymphocytes ($p \leq 0.01$)
- Increased densities of $CD3^+$, $CD4^+$, and $CD8^+$ T cells ($p \leq 0.01$) around vessels of the papillary loop in lesional skin.

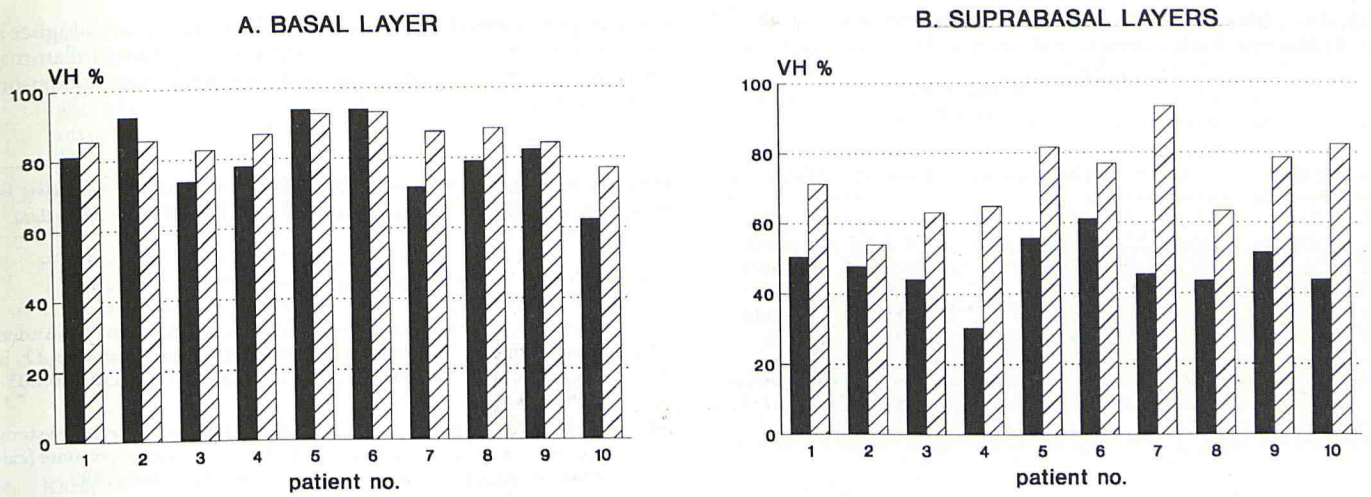

Figure 7. Increased proportion of VDR positively stained nuclei in lesional psoriatic skin in both the suprabasal and basal layer. *Bars*, VDR-positive area in relation to nuclear area (VH = A VDR/*A hematoxylin*) in percent. *Striped bars*, lesional; *solid bars*, non-lesional skin.

Table IV summarizes the mean expression of VDR by immunocompetent cells in the skin compartments I–III in normal and psoriatic skin. In lesional psoriatic skin the proportion of VDR-positive $CD3^+$T cells, $CD4^+$T-helper cells, and $CD11b^+$ macrophages was significantly increased compared to non-lesional skin ($p \leq 0.01$, $p \leq 0.05$, and $p \leq 0.01$).

DISCUSSION

The present study shows strong evidence that almost every cell type in normal human epidermis and dermis expresses VDR in vivo. In addition to epithelial cells, melanocytes were VDR immunoreactive, supporting a recent study on cultured melanocytes [10]. Earlier biochemical and experimental data pointed to the presence of specific binding sites for $1,25(OH)_2D_3$ in cells of the immune system [12,13] or skin [6,7,27,28]. Recent immunohistochemical studies [23,29] further suggested that the human skin might also be a target for $1,25(OH)_2D_3$ in vivo.

Up to now no data were available on the expression of VDR in Langerhans cells and granulocytes. Our findings raised the question as to what might be the significance and function of the VDR/$1,25(OH)_2D_3$ in these cells of the normal skin. VDR expression by Langerhans cells points to the relationship of this cell type to the monocyte/macrophage lineage that constitutionally expresses VDR. It would be interesting to know whether function and differentiation of Langerhans cells are modulated by $1,25(OH)_2D_3$ as well, as recently shown for monocytes and macrophages [12,13]. In vitro studies are also required to define the function of VDR expression in CD15/CD16-positive granulocytes.

Unexpected in our study was the high percentage of T lymphocytes heavily expressing VDR. T lymphocytes in normal skin seem to differ from T lymphocytes in peripheral blood. The latter express VDR only after activation in vitro [12,13] and with similar proportion in the two subsets, $CD4^+$ helper/inducers and $CD8^+$ suppressor/cytotoxic cells [30].

Epidermal keratinocytes, melanoma cells, and activated macrophages can convert 25-hydroxy vitamin D_3 [31–33] into the bioactive metabolite $1,25(OH)_2D_3$ in vitro. Accumulation of macrophages in the skin might contribute to increased local levels of $1,25(OH)_2D_3$. It is therefore tempting to speculate that $1,25(OH)_2D_3$ could play a role in normal differentiation and immune response in the skin.

Our primary hypothesis was that altered differentiation and ex-

Table III. Immunocompetent Cells in Different Compartments in Lesional and Nonlesional Psoriatic Skin[a]

		Compartment[b]					
		I		II		III	
CD		Non-Lesional	Lesional	Non-Lesional	Lesional	Non-Lesional	Lesional
$CD1a^+$	φ	43.0 ± 10.8	10.4 ± 4.3	20.3 ± 14.6	35.6 ± 7.1	97.7 ± 37.8	112.3 ± 64.4
	p	≤0.05		NS		NS	
$CD3^+$	φ	10.2 ± 13.8	23.1 ± 12.8	38.8 ± 33.0	156.9 ± 56.0	207.7 ± 34.9	262.5 ± 130.6
	p	≤0.01		≤0.01		NS	
$CD4^+$	φ	15.1 ± 7.0	15.6 ± 7.7	82.4 ± 57.8	157.7 ± 53.4	305.6 ± 154.3	307.5 ± 186.1
	p	NS		≤0.01		NS	
$CD8^+$	φ	1.9 ± 5.0	6.4 ± 4.7	5.8 ± 3.2	33.5 ± 15.2	80.3 ± 35.8	89.2 ± 40.3
	p	≤0.01		≤0.01		NS	
$CD11b^+$	φ	1.9 ± 5.1	19.3 ± 7.0	141.9 ± 49.9	174.1 ± 81.0	211.4 ± 36.4	235.8 ± 139.0
	p	≤0.01		NS		NS	
HLA-DR^+	φ	24.3 ± 5.1	20.7 ± 13.6	129.2 ± 59.5	229.2 ± 78.2	379.8 ± 50.0	329.8 ± 136.8
	p	≤0.05		≤0.05		NS	

[a] Expressed as number of CD^+ cells adjusted per 0.1 mm^2 section area.
[b] I, epidermis; II, perivascular papillary loop; III, perivascular superficial plexus; φ, mean ± SD; n = 8; NS, not significant.

Table IV. Mean VDR Expression by Immunocompetent Cells in Normal, Non-Lesional, and Lesional Psoriatic Skin[a]

VDR/CD	Normal Skin n = 5	Psoriatic Skin N = 8		Wilcoxon[b]
		Non-Lesional	Lesional	
$VDR^+/CD1a^+$	63.2 ± 14.1	75.1 ± 12.1	70.1 ± 10.6	NS
$VDR^+/CD3^+$	58.2 ± 17.0	52.1 ± 12.0	65.1 ± 7.5	$p \leq 0.01$
$VDR^+/CD4^+$	62.6 ± 4.7	64.1 ± 7.1	71.0 ± 8.7	$p \leq 0.05$
$VDR^+/CD8^+$	66.7 ± 8.2	64.1 ± 16.5	71.6 ± 11.9	NS
$VDR^+/CD11b^+$	50.2 ± 10.1	65.8 ± 7.3	75.5 ± 7.0	$p \leq 0.01$
$VDR^+/HLA\text{-}DR^+$	59.8 ± 9.6	66.4 ± 9.8	70.3 ± 6.2	NS

[a] Expressed as mean percentage of VDR positive cells in relation to total CD positive cells ± SD; three non-overlapping fields for each of the three skin compartments I–III were analyzed.

[b] Wilcoxon matched pairs signed rank test (non-lesional versus lesional psoriatic skin).

cess proliferation in psoriasis might be attributed to a (genetically) reduced VDR expression in the skin. In contrast, we found a significant increase in VDR immunoreactivity in epidermal keratinocytes and cells of the skin immune system in lesional as compared to non-lesional psoriatic skin. Our results do not allow any conclusion upon the function of the immunoreactive VDR. The apparent resistance of psoriatic skin to $1,25(OH)_2D_3$ as observed in some in vitro experiments [16,17] could also be explained by increased catabolism of $1,25(OH)_2D_3$ or a mild genetic defect in psoriatic patient VDR that cannot be revealed by immunohistochemistry. In other words, the immunoreactive epitope on VDR might be unaffected whereas the hormone-binding or DNA-binding domains or other domains participating in transcriptional control or post-receptor signalling could be altered.

How do our present findings fit into the current pathophysiologic concept of psoriasis or $1,25(OH)_2D_3$/ VDR biology? Psoriatic keratinocytes were shown to display a shortened cell cycle with an increased number of epidermal cells in the proliferative pool [34,35]. It appears that VDR expression may be a function of the state of differentiation. In various cell cultures VDR displays highest maximal ligand binding in the early logarithmic phase of cell growth, and diminishes as cells reach confluence [8,28]. Our finding of increased $1,25(OH)_2D_3$ receptor expression in lesional psoriatic keratinocytes could reflect the high proliferative activity and altered differentiation pattern in psoriasis. It is accepted that the steroid hormone responsiveness is directly proportional to the receptor number [36]. The finding that lesional cells express higher VDR immunoreactivity would explain why lesional skin responds to therapeutic doses of the hormone, whereas uninvolved areas remain unaffected.

Recent work emphasized the importance of the immunologic mechanism involving release of cytokines by activated T cells and macrophages in the pathogenesis of psoriasis [14,15]. It is not yet known whether cytokines modulate VDR expression in keratinocytes or leukocytes.

Activated macrophages were shown to produce $1,25(OH)_2D_3$ by an inducible 25-hydroxyvitamin D-1-hydroxylase and it was postulated that locally released $1,25(OH)_2D_3$ might suppress activation and recruitment of T cells [12]. $1,25(OH)_2D_3$ inhibits the transition of T lymphocytes from early to late G1 phase and acts as potent inhibitor of Il-2 and interferon-gamma (IFN-gamma) production in these cells [39]. Macrophages can be stimulated to increase their synthesis of 25-hydroxyvitamin D-1-hydroxylase by IFN-gamma via products of the 5-lipoxygenase pathway [40]. Increased $1,25(OH)_2D_3$ synthesis by local macrophages may represent a negative feedback mechanism counterbalancing further T-cell activation and pro-inflammatory release of IFN-gamma and leukotriene C4. Because $1,25(OH)_2D_3$ was shown to upregulate its own receptor [37,38], an increased VDR immunoreactivity in epidermal and dermal cells in psoriatic lesion might reflect higher local levels of the hormone. Topical treatment with $1,25(OH)_2D_3$ or analogues is suggested to enhance such immunosuppressive and anti-inflammatory pathways, which seems to be ineffective under untreated psoriatic conditions.

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